OSMOTIC REGULATION IN MARINE AND FRESH-WATER GAMMARIDS (AMPHIPODA)[1,2]

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Different species of the amphipod genus *Gammarus* live in habitats with a wide range of salt concentrations, from fresh water to sea water. To withstand such diverse conditions, the species must differ at least quantitatively in their osmotic physiology. In comparing four British species of the genus, Beadle and Cragg (1940a) found that the salinity range which each species tolerated correlated with the level at which it regulated the chloride concentration and the total osmotic concentration of its blood. More recently, Shaw and Sutcliffe (1961) and Lockwood (1961) have analyzed some mechanisms responsible for the osmoregulatory differences between two of Beadle and Cragg's species.

The present paper, too, attempts to analyze osmoregulatory mechanisms of gammarids, using an approach rather different from those of the previously mentioned investigators. The work involves four previously unstudied species: three in the genus *Gammarus* and one in the closely related genus *Marinogammarus*. The species are first compared with respect to the blood concentrations which they maintain in various dilutions of sea water. Then the role of the nephridium in salt and water excretion is studied in two species: one marine and one fresh-water. The results obtained imply some specific differences in the ionic uptake mechanisms.

Experimental Material

Adult males of the following four species were used:

Gammarus oceanicus Segerstråle (1947), characterized in the original description as "mainly marine," is widely distributed along both shores of the more northern parts of the Atlantic Ocean. It also lives in brackish situations and has been found in the Gulf of Finland in salinities as low as 2.5‰. Originally described as a subspecies of *G. zaddachi* Sexton, it was elevated to specific rank by Kinne (1954). The animals used in my experiments (mostly 18 to 23 mm. in length and 110 to 220 mg. in weight) were collected from an intertidal fresh-water seep on Cape Cod Bay, about two miles north of the Cape Cod Canal.

[1] Presented to the Faculty of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Contribution number 1291, Woods Hole Oceanographic Institution.

[3] Part of the work reported here was undertaken while I was recipient of a National Science Foundation Graduate Fellowship, and part while I was research assistant at the Woods Hole Oceanographic Institution. I wish to express special indebtedness to my thesis director, Professor G. E. Hutchinson, whose suggestions led to the present researches, and to Mr. H. J. Turner, who made available the facilities of his laboratory at the Woods Hole Oceanographic Institution and who aided the work in innumerable ways.

Marinogammarus finmarchicus (Dahl) was characterized by Sexton and Spooner (1940, p. 659) as "a marine littoral species, occurring intertidally along the sea coast and for a short distance up estuaries." The genus was formerly (Schellenberg, 1937) a subgenus of *Gammarus*. The animals (about 15 to 20 mm. long) were collected from a rocky shore at Manomet, Massachusetts, on Cape Cod Bay.

Gammarus tigrinus Sexton (1939) is a brackish-water species very similar in appearance to *G. fasciatus*. For a time the two names were considered synonymous. That the forms are actually separate species is indicated by the following evidence: there are some slight but consistent morphological differences (Bousfield, 1958); the osmotic physiology of the two forms differs, as shown later in this paper; and in the culture experiments I performed, the two bred separately but did not cross-breed. The experimental animals came from a fresh-water seep high in the intertidal zone on Nonamesset Island, in Vineyard Sound, Massachusetts. They were 10 to 13 mm. long.

Gammarus fasciatus Say is the common fresh-water species in New England and was taken from various streams near Woods Hole, Massachusetts, and New Haven, Connecticut. The animals were 10 to 15 mm. in length and 25 to 60 mg. in weight.

Methods

Determining osmotic concentration

The osmotic concentration of fluids was determined from the freezing point depression. Actually, the melting point of solutions was determined by observing a frozen sample as it was slowly warmed.

The apparatus used is a modification of the one designed by Kinne (1952) and is also similar to one devised by Ramsay (1949). It consisted of an aquarium of copper and glass which was mounted in the front wall of an insulated box and filled with a 25% solution of ethyl alcohol in water. The continuously stirred bath was warmed by an immersion heater, which was regulated by a variable transformer. The bath was cooled by a pack of crushed dry ice, which was pressed against the back of the aquarium by a spring-driven plunger. By careful balancing of the heat source and sink the temperature could be accurately controlled. The temperature was read with a Heidenhain thermometer graduated to 0.01° C.

To obtain a blood sample, the animal was gently dried off between layers of absorbent paper toweling, and its back was wiped with filter paper moistened with distilled water. With the animal held under paraffin oil the exoskeleton over the heart was punctured with a fine needle. From the drop of blood which appeared, a sample was taken with a fine Pyrex capillary tube, which had been cleaned in hot nitric acid. First, paraffin oil was drawn up into the capillary, then a small sample, and finally more paraffin oil. About a centimeter of capillary containing the sample was broken off, the ends sealed with sealing wax, and a label affixed. The samples were stored in a freezer. The size of the sample drops was not precisely controlled, but usually was 0.1 to 0.2 mm. in diameter and 0.3 to 1 mm. in length. This gave a range in volume from about 0.003 to 0.03 mm^3.

To determine the freezing point the sample was first frozen in crushed dry ice and then transferred to the controlled temperature bath, which had been cooled

below the expected freezing point of the sample. As many as six samples were held simultaneously in the bath by a movable clamp. The samples were illuminated by light passed through a square of Polaroid film and reflected from a mirror behind them. They were watched through the front glass wall of the aquarium with a horizontally mounted microscope containing a Polaroid analyzer. Since ice crystals are birefringent, use of polarized light made the crystals easier to see as brilliant white objects against a dark background.

The temperature of the bath was raised fairly rapidly until most of the ice in the sample had melted. The rate of temperature rise was then slowed to 0.01° C. per minute, or less. At this slow rate it could be assumed that the ice and the melted solution were nearly in thermodynamic equilibrium, and that the last crystal disappeared at the freezing point, provided that the rate of temperature rise had been slowed five minutes or more before this event. The method was accurate within 0.01° C. on standard salt solutions. In duplicate blood samples from a series of animals the maximum difference in the freezing point was 0.02° C.

Since a major concern was with the osmotic movement of water, it was deemed most suitable to express the freezing point depressions and the salinities of the media as moles of ideal non-electrolytic solute per kilogram of water. The equivalent in moles of monovalent salt, such as NaCl, may be found by dividing by 2. Use of molal units has the additional convenience that the osmotic concentration of "normal" sea water (chlorinity of 19.4‰) is nearly unity: 1.03 molal. Hence the molality of a solution is nearly equal numerically to its fraction of sea-water strength.

Determining osmoregulatory behavior of species

Animals were individually isolated without food in about 200 ml. of medium in one-pint polyethylene boxes during the period of adaptation to a new salinity. It was experimentally determined that the blood concentration of *Gammarus oceanicus* reached a new steady-state about 12 hours after transfer from undiluted sea water (0.93 molal at Woods Hole) to 0.1 molal sea water. *G. fasciatus* reached a new steady-state within 1½ hours after transfer from fresh water to 0.6 molal sea water, the highest concentration in which it normally survived. Although adaptation times to other salinities were not determined, these experiments are taken to indicate that the one- or two-day period of adaptation was appropriate.

Animals were transferred to fresh water and to sea water of molality 0.03, 0.1, 0.2, 0.4, 0.61, 0.82, 0.93 or 1.03, and, in some instances, 1.5. (These concentrations correspond to salinities of 1.0, 3.5, 7.0, 14, 21, 28, 32 or 35, and 51.5‰.) All the species were exposed to the experimental media for 48 hours, or a little longer, with the exception of *G. oceanicus,* which was exposed for only 24 hours. Owing to the method of sampling, each animal could be used only once; hence the data shown on the curves are composite. The total number of surviving animals, distributed more or less equally among the various media, was 51 for *G. oceanicus,* 74 for *M. finmarchicus,* 77 for *G. tigrinus,* and 64 for *G. fasciatus.* The temperature range during the experiments was 16 to 19° C. for *G. oceanicus,* 14 to 16° C. for *M. finmarchicus* and *G. tigrinus,* and 13 to 18° C. for *G. fasciatus.* The curves were determined in July, 1955, for *G. oceanicus,* in September, 1955, for *G. fasciatus,* and in July, 1956, for *G. tigrinus* and *M. finmarchicus.*

No attempt was made to determine or control the molting stage of the experi-

mental animals. Baumberger and Olmsted (1928) found that the concentration of the blood in some brackish-water crabs doubled during a molt. In my experiments the blood concentration in any one medium varied only slightly. Either a concentration change does not occur during molt in these animals or it is transitory and was not encountered.

Determining urinary rate and concentration

The structurally simple nephridium of *Gammarus* has an end-sac and a more or less coiled canal but no storage bladder (Burian and Muth, 1924; Schwabe, 1933). This arrangement should result in a continuous flow of urine, rather than intermittent micturition. The nephridium opens at the tip of a protuberance (the nephrocone) on the second segment of the second antenna; consequently the opening is readily accessible.

Sampling the urine was first attempted by immersing the animal in paraffin oil and, with a capillary tube, picking up drops of urine formed at the nephropore. This method was satisfactory for getting urine samples for freezing point determination, but not for finding rates of urine flow. The animals died after a short period under these conditions, presumably because the water clinging to the gills rapidly became anaerobic.

To overcome this difficulty the following arrangement was devised. A short, bent piece of glass tubing was cemented to the bottom of a Petri dish, with one end opening horizontally and the other end vertically. The dish was filled with water of the desired concentration to a depth that covered the horizontal end of the tubing. The animal was removed from the adapting medium, grasped firmly by the coxal plate of the first right thoracic leg with watchmaker's forceps, and backed gently into the open end of the tube. The forceps was then clamped in place. The coxal plate is extremely thin, but is broad and usually is quite hard and sturdy. Grasping it had no apparent effect upon the rate of urine flow. Various diameters of glass tubing were used so that the animals of different sizes could be fitted snugly. Thus, although the animal was held firmly at only one point, its movement was greatly restricted and it could not rotate about that point. After the animal had been introduced into the tube and had quieted, the water surrounding the tube was removed and replaced with paraffin oil. Surface tension held the water in the tube during the change, which left the animal with most of its body in water and only its head protruding into the oil. The water was aerated by a bubbler made of fine tubing inserted into the upper end of the tube.

Once the head and the nephrocones on the second antennae were surrounded by oil, it was possible to collect urine. For this purpose micropipettes were made from 0.3- or 0.5-mm. bore capillary tubing, tapered at the tip and graduated with strips of millimeter graph paper. The pipettes were filled with oil to avoid a strong capillary pull. They were then immersed in the oil bath with their tips capping the nephrocones. In some cases, especially with the smaller animals, the surface tension at the oil-urine interface of the minute nephridial opening apparently was sufficient to prevent the urine from flowing. To start the flow, it was usually necessary merely to touch the inside wall of the pipette to the nephropore and thereby break the interface. The rate of urine flow was determined from the progress of the urine drop up the pipette. Both nephridia were sampled simultaneously;

their rates rarely differed by as much as a factor of two. Urine flow was measured for about half an hour, a reading of the level in the pipettes being taken every five minutes in most cases, every two minutes in some others. The mean rates of urine flow for each side were added to give the total rate.

One difficulty was encountered with this method. Although most animals produced a measurable amount of urine when in any of the lower salinities, some did not. In most cases these animals had been injured in handling. Recently molted animals with soft exoskeletons were especially prone to damage. The results from

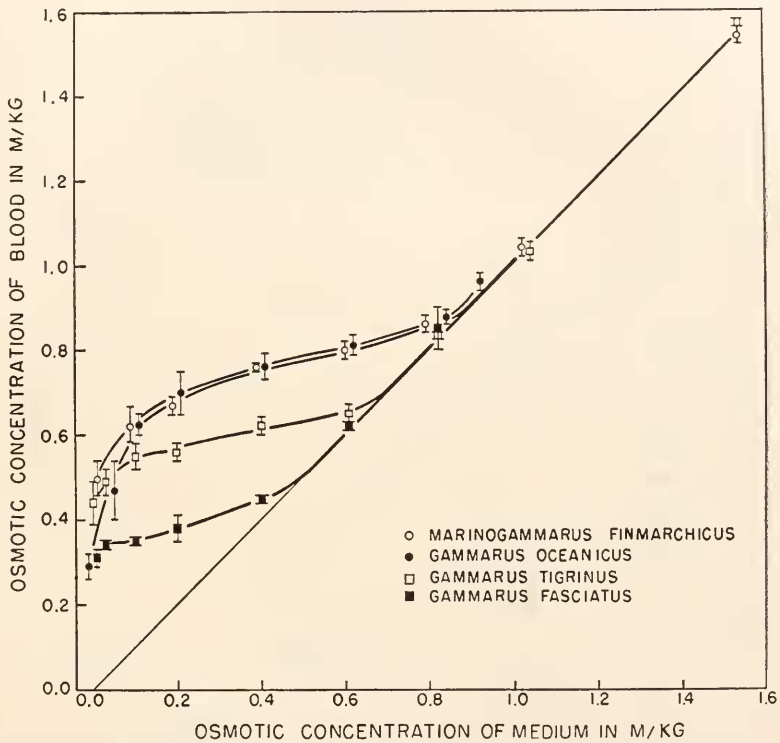

FIGURE 1. Relationship of concentration of blood (in moles of ideal solute per kg. of water) to concentration of medium in *Marinogammarus finmarchicus* and *Gammarus oceanicus* (marine), *G. tigrinus* (brackish-water), and *G. fasciatus* (fresh-water). Points represent means; vertical bars represent ±2 × the standard errors.

damaged animals were discarded. There were a few cases of no urine flow for which there was no observed damage. Data from these animals are included in the graphs but not in the statistical calculations.

The choice of media was determined by the desire to find the relation of the rate of urine flow not only to the external concentration, but also to the osmotic gradient (obtained by subtracting the sea-water concentrations from the blood concentrations in Figure 1). The media chosen were ones in which a wide range of gradients would be expected: 0.03, 0.2, 0.61, and 0.93 molal sea water for the marine species *G. oceanicus,* and fresh water, 0.2, and 0.61 molal sea water for

the fresh-water *G. fasciatus*. The experiments were carried out at approximately 15° C.

Results

Osmoregulatory behavior

The four species regulated osmotically in the manner shown in Figure 1. Each point is the mean of determinations on several animals (see methods section). The vertical lines at each point give ±2 × the standard error of the mean, or the 95% confidence interval for the mean.

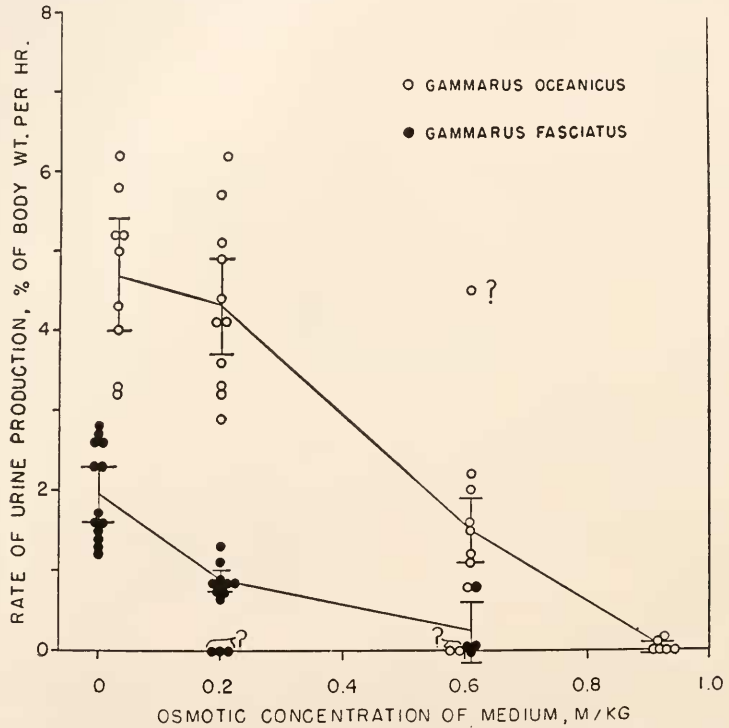

Figure 2. Relationship of rate of urine flow to external concentration in *Gammarus oceanicus* (marine) and *G. fasciatus* (fresh-water). Points represent individual animals. Points marked with a question mark are not included in the statistics. Curves connect the means. Vertical lines show ±2 × the standard errors.

The marine species (*M. finmarchicus* and *G. oceanicus*) exhibited nearly identical relationships of blood concentration to external concentration. In full strength sea water their blood was nearly isotonic to the medium. They began to regulate the blood concentration when sea water was diluted only slightly to below 0.85 molal. As the medium was further diluted, they maintained a progressively greater concentration gradient, and in 0.2 molal sea water the blood concentration was still 0.7 molal. In the most dilute sea-water media the gradient failed to increase further, or even decreased, but all animals in 0.03 molal sea water survived. In fresh water all *M. finmarchicus* died, but three out of ten *G. oceanicus* survived 24 hours,

or long enough to be sampled, although their condition was probably moribund. In another experiment no *G. oceanicus* survived even this long in fresh water.

The brackish-water *G. tigrinus* displayed an osmotic behavior intermediate between that of *G. oceanicus* and *M. finmarchicus* on the one hand and that of *G. fasciatus* on the other. It regulated in media of less than 0.65 molal concentration and kept the blood concentration at 0.55 molal in 0.1 molal sea water and at 0.44 molal in fresh water. The most remarkable aspect of this experiment is that the

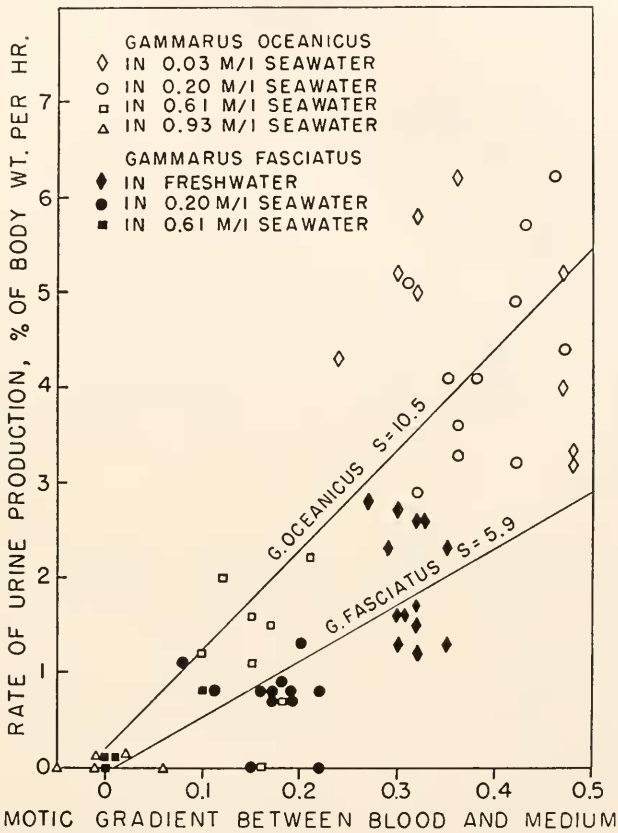

FIGURE 3. Relationship of rate of urine flow to osmotic gradient between blood and medium in *G. oceanicus* and *G. fasciatus*. Slope (S) determined by method of least squares.

species survived after direct transfer both to fresh water and to 1.5 molal sea water, although its mortality in either of these extreme concentrations was about 50%. In other experiments, some individuals survived a period of weeks in fresh water.

G. fasciatus had a blood concentration of a little over 0.3 molal in fresh water, its normal habitat. When the animals were transferred into dilutions of sea water, the blood concentration increased only slightly and became isotonic in media with a concentration more than 0.5 molal. The species survived well in 0.6 molal sea water, but most individuals in 0.8 molal and all in 1.0 molal sea water died.

Urinary rate

Rate of urine flow is plotted against concentration of medium in Figure 2. Despite considerable variability in the data, it is apparent that in both species the rate of flow was much greater in the more dilute media. Urine production was

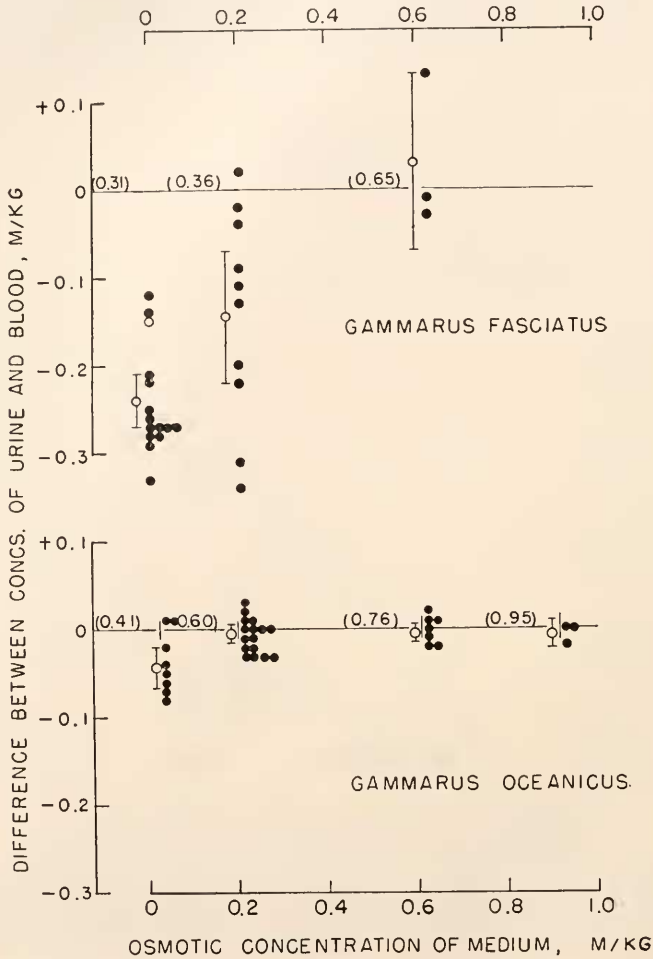

FIGURE 4. Relationship of urine concentration to blood concentration in various media, in *G. oceanicus* and *G. fasciatus*. Filled circles represent the difference: (urine concentration minus blood concentration) for individuals. Open circles show the mean difference; vertical lines $\pm 2 \times$ the standard errors. Mean blood concentrations are given in parentheses.

detectable at higher salinities in *G. oceanicus* than in *G. fasciatus*. Moreover, the maximum rate (over 4% of the body weight per hour) in *G. oceanicus* was more than twice as great as the maximum rate (around 2%) in *G. fasciatus*.

A few determinations on specimens of both species at 25° C. indicated that the rate was roughly twice that of animals in the same concentrations at 15° C.

The rate of urine flow in the two species is plotted against the osmotic gradient in Figure 3, and the regression lines are shown. The correlation coefficients for these data were 0.84 for *G. oceanicus* and 0.75 for *G. fasciatus*. In both cases the probability that a coefficient this size arose by chance is less than 0.001, as determined by a t-test. The slopes or regression coefficients show that the best estimate of the rate of urine production was 10.5% of the body weight per hour per molal gradient in *G. oceanicus* and 5.9% in *G. fasciatus*. If the regression coefficients are compared by a t-test presented by Fisher (1950), it is found that the probability they were drawn from the same statistical population is approximately 0.02.

Urine concentration

The difference between the urine concentration and the blood concentration for the two species of *Gammarus* in different salinities is shown in Figure 4. The urine of *G. oceanicus* was approximately isotonic to the blood in all media except the most dilute. In the latter, it was hypotonic by a small but statistically significant amount. The urine of *G. fasciatus* in fresh water was strongly hypotonic to the blood. In 0.2 molal sea water there was a great deal of variability, the urine ranging from equal in concentration to the blood to more dilute than the medium. The data for 0.6 molal sea water unfortunately are rather inadequate, partly because of difficulty in getting enough urine for a freezing point sample and partly because one of the samples seems to have been contaminated. However, the urine at this salinity appeared to be isotonic with the blood.

Discussion

Role of nephridium

Since the rate of water movement across a semipermeable membrane is proportional to the osmotic gradient, the demonstration that the urinary rate is proportional to the concentration difference between blood and medium supports the hypothesis that the water which enters *Gammarus* by osmosis is eliminated as urine. However, in order to prove the hypothesis completely, it would be necessary to measure the osmotic uptake of water by some independent method, and to demonstrate that urine flow is adequate to account for the removal of this water. Although this plausible mechanism of water balance in crustaceans has often been proposed before, the evidence regarding both the proportionality of urine flow and its adequacy has not been conclusive. The evidence on each of these points will be considered in turn in the following paragraphs.

In most studies of urinary rate in crustaceans and its relation to the external concentration, the method has been to plug the nephridial opening and to assume that subsequent weight increases represented normal urine formation. This procedure is suspect *a priori* in an armored animal such as a crustacean. One would expect blocking the nephridial opening to cause an increase in the hydrostatic pressure in the nephridium and, if urine formation occurs through filtration, to cause a change in the rate of this process. Indeed, plugging the kidneys has been reported as leading to the death of the experimental animals by Herrmann (1931) in *Astacus;* and by Nagel (1934) in *Carcinus*. While this method has sometimes yielded results in accord with those from other methods (see discussion of Parry's

work, below), it has also given ambiguous results. For example, Nagel (1934) found that when crabs with plugged nephridia were transferred to brackish water they gained slightly more on the average than did similar crabs left in sea water. However, t-tests upon Nagel's data show the difference between transfer and control groups was of dubious statistical significance: $p > 0.2$ for the difference between rates for two groups given the same period of exposure; $p = 0.05$ for the difference between rates for two groups given different exposure times.

The most thorough study of the influence of salinity variation upon the rate of urine production in crustaceans was made by Parry (1955) in the prawn, *Palaemonetes varians*. This investigator estimated the rate of urine flow from four independent measurements: (1) the time for clearance of injected dye, (2) the frequency of *micturition* from a bladder of known size, (3) the weight change after blockage of the nephropores, and (4) the volume of urine collected by cannulation of one of the nephropores. All four methods gave similar results. Parry did not relate the rates to the gradient between blood and medium, since she did not

TABLE I

Relation between osmotic gradient and rate of urine flow in Palaemonetes varians

Osmotic conc. of medium (moles/kg.)	Gradient between blood and medium (calculated from Panikkar, 1941)	Urine flow as % of body wt./hr. (determined by rate of micturition, Parry, 1955)	Ratio: $\left(\frac{\text{Urine flow}}{\text{gradient}}\right)$
.05	.49	1.63	3.3
.15	.39	1.06	2.7
.25	.29	.94	3.2
.50	.07	.15	2.1
.67	−.10	.45	−4.5
.85	−.25	.40	−1.6
1.00	−.34	.42	−1.2
1.20	−.51	.11	− .22

determine blood concentrations. However, if we use concentrations determined for the same species by Panikkar (1941; also cited by Parry), we can consider the data from this viewpoint (Table I). We see that during hypertonic regulation in external concentrations of less than about 0.6 mole/kg., the rate of urine production was approximately proportional to the osmotic gradient. It is conceivable that the small increase in urinary rate during hypotonic regulation has the same cause as the increased urinary rate in injured marine teleosts (Smith, 1932), namely, the necessity of excreting the extra divalent ions in swallowed sea water.

The rate of water entry into crustaceans has not been determined with sufficient precision to permit an exact comparison with the rate of urine flow. In the previously mentioned study, Parry found that the half-time for penetration of heavy water into *P. varians* in nearly isotonic conditions was one-half to three-fourths hour. Such determinations may be used (*cf.* Lockwood, 1961) to predict the rate at which water should be absorbed under a specified osmotic gradient. Treating Parry's data in this manner, one may calculate that an amount equaling 1.9% to 2.5% of the total body water (or 1.5% to 2.0% of body weight, if a prawn is 80% water) should enter per hour and per gradient of one mole/kg. The values for

urinary rates in column 4, Table I, are roughly comparable (2.1% to 3.3% of body weight per hour and per gradient of one mole/kg.).

Lockwood (1961) has determined the rate of penetration of tritiated water into two British species of *Gammarus: G. pulex* and *G. duebeni.* The difference between the two species was not statistically significant. The half-time for penetration in *G. duebeni* was 13.9 minutes, which leads to an estimated rate of water entry of about 4.7% of body weight per hour per gradient of 1 mole/kg. While this is less than the rates of urine flow shown by the slopes in Figure 3, it is of the same order of magnitude.

In summary, the similarity of observed urinary rates to those predicted from permeability studies is at least in accord with the view that osmotically absorbed water is eliminated as urine. The data are not sufficiently exact, however, to be decisive.

Comparison of species

A. Comparison of *G. fasciatus* and *G. oceanicus*

Three differences have been shown which give the fresh-water *G. fasciatus* a lower rate of urinary salt loss than the marine *G. oceanicus.* They are (1) a smaller concentration gradient between blood and medium, (2) a lesser permeability to water,[4] and (3) a urine which is hypotonic to the blood.

The first two differences result in a lower rate of urine flow; the third results in a smaller salt loss for a given volume of urine. The quantitative result of these differences is shown in Figure 5, in which the urinary rate is multiplied by the urine concentration to give the rate of salt loss in micromoles of solute particles per hour in a 100-mg. animal.

B. Comparison with other species

None of the species in this study was the same as any in the study by Beadle and Cragg (1940a). Two of their species are not known to occur at all in North America, and the other two do not occur in southern New England, where the present study was made. However, Beadle and Cragg's findings were similar to those presented here in that their more marine species, *G. locusta* and *G. (Marinogammarus) obtusatus,* regulated the blood concentration at a high level; the brackish-water *G. duebeni* regulated at an intermediate level; and the fresh-water *G. pulex,* at a low level. The major differences from the present study were that their marine species, especially *G. obtusatus,* did not survive in nearly as dilute sea water as did *G. oceanicus* and *M. finmarchicus;* and that none of their species could survive in both fresh water and full strength sea water. (Later, however [1940b], Beadle and Cragg reported on a fresh-water race of *G. duebeni.*)

The recent papers of Lockwood (1961) and of Shaw and Sutcliffe (1961) were concerned largely with water entry, which has already been discussed, and

[4] From the difference in slopes in Figure 3. The rates of urine flow in this figure are based on the weights of the animal, whereas permeabilities should be based on the surface areas, which are not known. It can easily be shown, however, that since *G. oceanicus* averaged about five times the weight of *G. fasciatus,* conversion of urinary rate to permeability would *increase* the difference found between the species.

with sodium uptake, which will be discussed in the next section. Several aspects of the salt loss remain to be compared. Shaw and Sutcliffe found that the rate of salt loss by all routes from a 40-mg. *G. duebeni* varied from 0.17 to 0.76 micromole of NaCl per hour, being less when the animal had been adapted to a more dilute medium. For *G. pulex,* the rate was 0.09 to 0.18 micromole per hour.

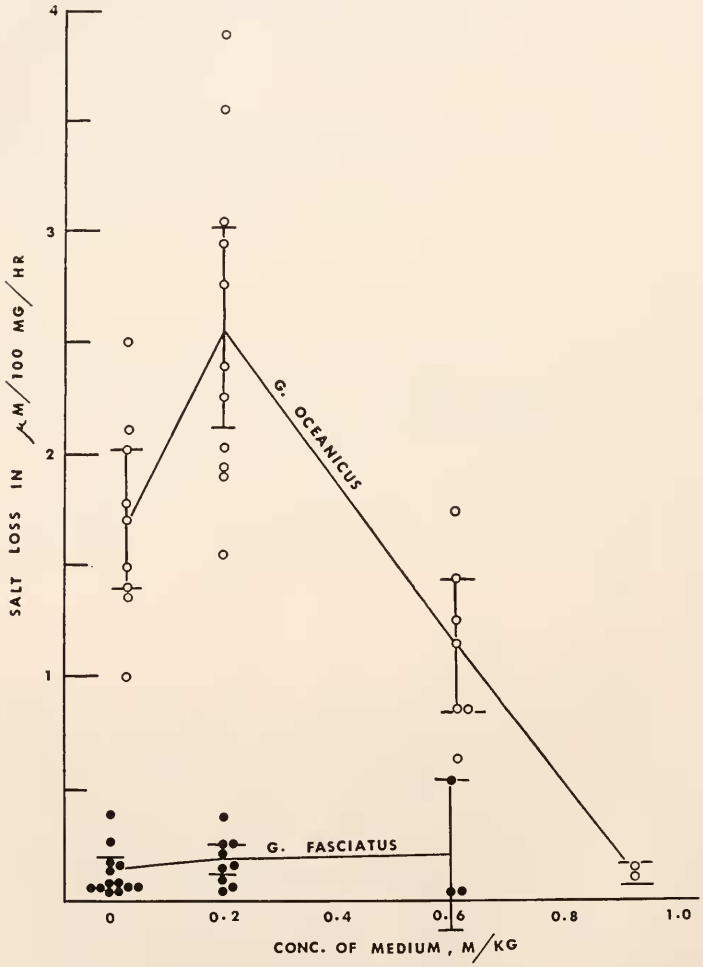

FIGURE 5. Comparison of rate of urinary salt loss in *G. occanicus* and *G. fasciatus.* Points represent individuals. Curves connect means. Vertical lines show ±2 × the standard errors.

Lockwood determined that in very dilute sea water both the brackish-water *G. duebeni* and the fresh-water *G. pulex* formed urine hypotonic to the blood. In the higher concentrations of sea water the urine of *G. duebeni* became isotonic to the blood, but the urine of *G. pulex* remained hypotonic to the blood and also to the medium. Estimating the urine flow from the penetration of tritiated water, Lockwood calculated that only 0.10 micromole per hour of the NaCl loss from *G. duebeni*

could be accounted for by the urine. Apparently there is a substantial loss by some other route, probably across the body surface.

From Figure 5 one can calculate that a 40-mg. *G. oceanicus* in the most dilute sea water would retain salt less efficiently than *G. duebeni* and would lose about 0.34 micromole of NaCl per hour through the urine alone. A *G. fasciatus* of the same size in fresh water would lose only about 0.02 micromole per hour.

Mechanism of salt uptake

The differences demonstrated between *G. fasciatus* and *G. oceanicus* bring to the fore a curious problem. The steady-state osmotic gradient maintained by these animals represents the conditions at which salt loss by all routes just equals salt uptake. Since the urine concentration and flow rate are more favorable for osmotic regulation in *G. fasciatus* than in *G. oceanicus*, how can the latter maintain a much greater osmotic gradient when both species are in the same medium? One logical possibility is that *G. fasciatus* is more permeable to salt than *G. oceanicus* and loses more salt by diffusion. This would be contrary to the results of other investigations of the comparative permeabilities of marine, brackish-water, and fresh-water crustaceans (Gross, 1957; Bethe, 1930; Nagel, 1934). The only alternative explanation of the apparent paradox is that when both species are in the same medium, the rate of salt uptake is greater in *G. oceanicus*.

From the steady-state condition it is apparent that when a gammarid is in a high external concentration, the mechanism for salt uptake must be operating either not at all or at only a very low rate, since the gradient is zero. The gradient becomes appreciable—that is, uptake begins—only when the concentration is brought below a certain critical level. Activation of the mechanism must be gradual, since the osmotic gradient increases gradually as concentration is further lowered. The critical concentration at which uptake and regulation begin is characteristic of the species, being highest in the marine species (*G. oceanicus* and *M. finmarchicus*), next highest in the brackish-water species (*G. tigrinus*), and lowest in the fresh-water species (*G. fasciatus*). Consequently, in most salinities the uptake mechanism is more completely activated in *G. oceanicus* than in *G. fasciatus* and the former species maintains a greater gradient. The degree of activation of the uptake mechanism might depend upon either the concentration of the medium or that of the blood. The latter alternative is favored by Shaw's (1959) demonstration that in the crayfish, sodium uptake from fresh water increases with decreasing blood concentration.

In most dilute media the gradient maintained by a species falls below the maximum, indicating a drop in the salt uptake rate. In this circumstance *G. oceanicus* no longer has a gradient greater than *G. fasciatus;* indeed, in fresh water it cannot maintain an internal concentration sufficiently high for survival.

Shaw and Sutcliffe (1961) directly measured uptake by *Gammarus* from very dilute media and found that with increasing external concentration the uptake rate rose asymptotically to a maximum. Since this behavior can be described by the Michaelis equation for effect of substrate concentration on rate of an enzyme-intermediated reaction, the implication is that in very dilute media the uptake mechanism becomes unsaturated. Using this interpretation, Shaw and Sutcliffe further concluded that the uptake mechanism of *G. pulex* had a greater affinity for

ions than that of *G. duebeni,* and therefore the former could maintain a greater gradient in the most dilute media. This analysis would equally well explain the difference in osmotic behavior between *G. fasciatus* and *G. oceanicus.*

Summary

1. The relationship of the osmotic concentration of the blood to that of the external medium is described for four species of gammarid. In media of various concentrations each species regulates its blood concentration in a manner that reflects its natural habitat. The marine species, *Marinogammarus finmarchicus* and *Gammarus oceanicus,* regulate their blood concentration at the highest level; the brackish-water species, *G. tigrinus,* regulates at a lower level; and the fresh-water species, *G. fasciatus,* regulates at the lowest level. Moreover, *M. finmarchicus* and *G. oceanicus* die in fresh water; *G. fasciatus* dies in full-strength sea water; but *G. tigrinus* survives both in fresh water and in sea water up to at least 1.5 molal.

2. The rate of urine production in *G. fasciatus* and *G. oceanicus* is proportional to the osmotic gradient between blood and medium, indicating that urine formation represents elimination of osmotically absorbed water. The coefficient of proportionality is smaller in *G. fasciatus* (5.9% of body weight per hour per molal gradient) than in *G. oceanicus* (10.5%), indicating that the latter species is more permeable to water.

3. The urine of *G. oceanicus* is nearly isotonic to the blood in all media. The urine of *G. fasciatus* is much more dilute than the blood.

4. The differences in flow and in concentration of urine combine to give *G. oceanicus* a much greater rate of urinary salt loss than *G. fasciatus.*

5. The osmotic gradient maintained by each species varies in a way that indicates the animals have an ionic uptake mechanism which is gradually activated as the salinity is lowered. In all except the most dilute media, it appears that the mechanism is more completely activated and takes up salt more rapidly in *G. oceanicus* than in *G. fasciatus.*

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